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PAPERS COMMUNICATED TO THE CHEMICAL SOCIETY.

General, Physical, and Inorganic.

Physical Organic.

Organic.

(*g*) Barium fluoride gave a residue with a ratio $BaF_2 : SeF_4$ 1·40 : 1, suggesting that a compound is formed which is not very stable.

(*h*) Silver fluoride dissolved on warming (0·05 g. in 10 g. of solvent) and gave a white precipitate on cooling the solution. As the solvent was removed, however, it began to decompose: an examination at an intermediate stage suggested the formula $AgSeF_5$. After 1 hour at 100° in a vacuum the material was visibly decomposed and the ratio $AgF : SeF_4$ was as low as 1 : 0·26. The best intermediate analysis gave the ratio $AgF : SeF_2 = 1·3 : 1$.

(*i*) Calcium fluoride, practically insoluble, retained only a trace of tetrafluoride after excess had been removed.

3. *Other observations.* Selenium tetrafluoride is decomposed violently by water and attacks phosphorus as does the oxyfluoride. It is miscible in all proportions with sulphuric acid, alcohol, ether, and iodine pentafluoride, and dissolves appreciable quantities of carbon tetrachloride, chloroform, bromine, iodine, sulphur, and selenium. Selenium tetrafluoride dissolves and reacts with all the potassium halides and some of the higher metallic and non-metallic oxides such as phosphoric oxide, vanadium pentoxide, and chromium trioxide. These reactions are in the course of quantitative investigation.

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KING'S COLLEGE, NEWCASTLE-ON-TYNE, 1. [*Received, November 11th*, 1951.]

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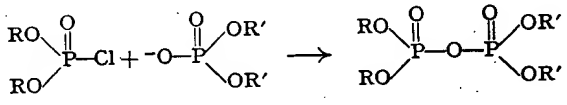
223. *Studies on Phosphorylation. Part X.* The Preparation of Tetra-esters of Pyrophosphoric Acid from Diesters of Phosphoric Acid by Means of Exchange Reactions.*

By N. S. CORBY, G. W. KENNER, and A. R. TODD.

The mechanism of the reaction between tetraphenyl pyrophosphate and dibenzyl hydrogen phosphate in presence of bases (Mason and Todd, *J.*, 1951, 2267) has been elucidated and the reaction has been extended to the preparation of other symmetrical and unsymmetrical pyrophosphates. The scope of this exchange reaction has also been extended to include the preparation of pyrophosphates by treating salts of diesters of phosphoric acid with arylsulphonyl halides and with trifluoroacetic anhydride.

ONE of the principal aims of current work in these laboratories is the synthesis of dinucleotide coenzymes (*e.g.*, cozymase, flavin-adenine-dinucleotide). The mononucleotides have been synthesised through intermediates in which the hydroxyl groups of phosphoric acids were blocked by benzyl groups (Baddiley and Todd, *J.*, 1947, 648; Baddiley, Michelson, and Todd, *J.*, 1949, 582; Michelson and Todd, *J.*, 1949, 2476, 2487; Brown and Todd, *J.*, 1952, 44). If the route to dinucleotide coenzymes is to be analogous, it will involve the preparation of unsymmetrical tetra-esters of pyrophosphoric acid from which two protecting benzyl groups will be removed in a later stage of the synthesis. The object of the present investigation has been to study on simple substances the preparation of tetraesters of pyrophosphoric acid by methods which might be applicable in the nucleotide field.

Hitherto unsymmetrical tetraesters of pyrophosphoric acid have always been prepared by reaction of a chlorophosphonate with the salt of a phosphate:



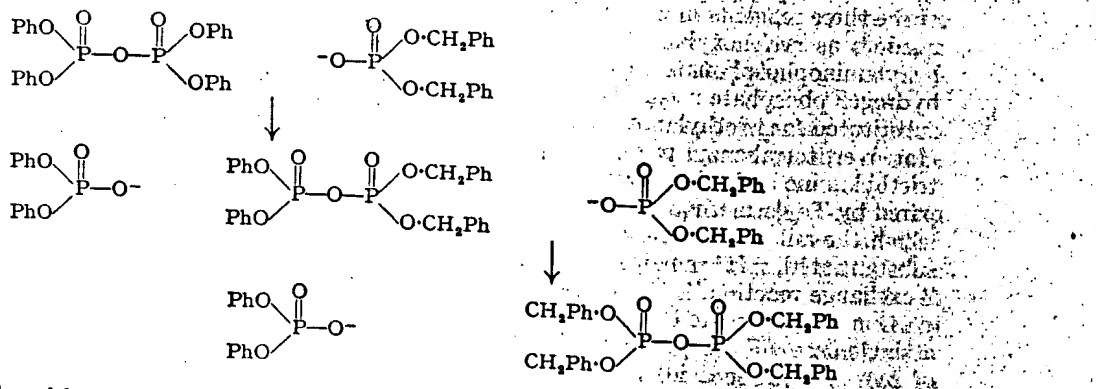
Thus the tribenzyl ester of adenosine-5′ pyrophosphate (ADP) has been prepared from dibenzyl chlorophosphonate and silver adenosine-5′ benzyl phosphate (Baddiley and Todd,

* Part IX, *J.*, 1951, 2271.

loc. cit.). Toy (*J. Amer. Chem. Soc.*, 1950, 72, 2065) has described the preparation of some simple unsymmetrical tetraesters by the analogous reaction of pyridinium salts and chlorophosphonates in ethereal solution. Now, whereas compounds such as adenosine-5′ benzyl phosphate and their salts are now relatively freely available, the corresponding chlorophosphonates are difficultly accessible for reasons already discussed in Part VIII of this series (Mason and Todd, *J.*, 1951, 2267). Recently we have indeed been able to prepare them, and will report upon them later, but the present work was devoted to consideration of alternative routes to pyrophosphates which do not entail the use of chlorophosphonates.

In Part VIII (*loc. cit.*) the direct conversion of simple diesters of phosphoric acid into their anhydrides by reaction with thionyl chloride and oxalyl chloride was reported; these reagents, proved quite unsatisfactory for work in the nucleotide field. The striking observation was, however, made (*loc. cit.*) that tetrabenzyl pyrophosphate could be prepared in high yield from dibenzyl hydrogen phosphate by reaction for a short time with tetraphenyl pyrophosphate at room temperature in anhydrous polar solvents in presence of a tertiary base. These conditions are well-nigh ideal for work with nucleotides and it was therefore important to examine more closely the nature and scope of the process. It was of particular interest to study the possibility of halting the reaction at an intermediate stage so as to obtain an unsymmetrical pyrophosphate.

The first point which we sought to establish about the nature of the conversion of dibenzyl hydrogen phosphate into tetrabenzyl pyrophosphate by means of tetraphenyl pyrophosphate was the function of the tertiary base. Accordingly the proportion of triethylamine added was varied in a series of experiments with one mol. of tetraphenyl pyrophosphate and two of dibenzyl hydrogen phosphate in acetonitrile solution. No tetrabenzyl pyrophosphate was isolated from reaction mixtures to which one mol. or less of triethylamine had been added. An 80% yield of pyrophosphate resulted when two mols. of base were used, but this fell with increasing quantities, being only 54% when the amount of base was increased to four mols. The obvious conclusion was that the base was required to form a salt of dibenzyl hydrogen phosphate. In confirmation two mols. of lithium dibenzyl phosphate were found equally effective as two of the free acid with two of triethylamine. These findings are in accord with the mechanism of a two-stage nucleophilic displacement, already mentioned in Part VIII (*loc. cit.*) and now set out in full below:



Two mols. of base are necessary to form the two mols. of dibenzylphosphate anion. It follows from this reaction scheme that the experiment conducted with only a single mol. of triethylamine should have led to the intermediate dibenzyl diphenyl pyrophosphate as well as triethylammonium diphenyl phosphate and dibenzyl hydrogen phosphate. The isolation of this unsymmetrical pyrophosphate as such from the reaction mixture would obviously be difficult, and therefore its independent preparation was investigated in order to facilitate its detection in the mixture.

As already mentioned, unsymmetrical pyrophosphates may be prepared by the interaction of silver dialkyl phosphates and chlorophosphonates. Indeed some years ago Dr. H. T. Howard (Thesis, Cambridge, 1946) prepared crude dibenzyl diphenyl pyrophosphate

from diphenyl chlorophosphonate and silver dibenzyl phosphate in acetonitrile solution. However the alternative procedure of Toy (*loc. cit.*) using pyridinium salts in benzene solution appeared cleaner and in fact was known to be preferable to the silver salt method (Mason and Todd, *loc. cit.*) for the preparation of tetraphenyl pyrophosphate (see Experimental section). When benzene solutions of pyridinium dibenzyl phosphate and diphenyl chlorophosphonate were mixed, precipitation of pyridine hydrochloride soon started. After three hours this was removed by filtration and the filtrate evaporated at a low temperature. An opalescent gum remained, which was insoluble in water but slowly hydrolysed during six hours in contact with water maintained at pH 8 by addition of dilute ammonia, producing only a little tetrabenzyl pyrophosphate. Addition of *cyclo*hexylamine to a benzene solution of the gum precipitated *cyclo*hexylammonium diphenyl phosphate; evaporation of the solution gave dibenzyl *cyclo*hexylaminophosphonate in an amount corresponding to 90% of the dibenzyl hydrogen phosphate used in its preparation. Clearly the mode of preparation and properties of this gum leave no doubt that it is almost entirely the desired mixed anhydride, dibenzyl diphenyl pyrophosphate. Its final purification by molecular distillation could not be achieved owing to the pyrolytic decomposition characteristic of benzyl esters of phosphorus oxy-acids. On the other hand, di-*p*-methoxyphenyl diphenyl pyrophosphate, prepared analogously from diphenyl chlorophosphonate and pyridinium di-*p*-methoxyphenyl phosphate, could be successfully distilled. During the distillation, however, it evidently disproportionated into tetraphenyl pyrophosphate and tetra-*p*-methoxyphenyl pyrophosphate, for on treatment with aniline it gave mixtures of anilinium salts and mixtures of anilinophosphonates. In contrast, the oil before distillation yielded only di-*p*-methoxyphenyl aminophosphonate on dissolution in liquid ammonia. The unsymmetrical tetraesters of pyrophosphoric acid should therefore be regarded as very susceptible to disproportionation and Toy's claims (*loc. cit.*) that they can be distilled should be regarded with reserve in the absence of further experimental data.

The preparation and knowledge of the properties of dibenzyl diphenyl pyrophosphate enabled us to get further insight into the reaction between dibenzyl hydrogen phosphate and tetraphenyl pyrophosphate. The reaction between two mols. of the former, one of the latter, and one of triethylamine led to an oil moderately stable to water. Addition of *cyclo*hexylamine to a benzene solution of the product of reaction between equimolar amounts of the three reagents in acetonitrile caused precipitation of 80% of the diphenyl phosphoric residues as *cyclo*hexylammonium diphenyl phosphate. From the liquors dibenzyl *cyclo*hexylaminophosphonate was obtained in amount corresponding to 72% of the dibenzyl hydrogen phosphate used. A similar reaction in dimethylformamide, with *cyclo*hexylamine substituted for triethylamine, gave an 81% yield of dibenzyl *cyclo*hexylaminophosphonate. Moreover, tetrabenzyl pyrophosphate was produced on addition of equimolar amounts of triethylamine and dibenzyl hydrogen phosphate to the unsymmetrical pyrophosphate prepared by Toy's method. In our view the mechanism written above for the reaction in which the anhydride condition is exchanged between diesters of phosphoric acid is fully substantiated. It remains to consider the factors controlling the direction of this "exchange reaction."

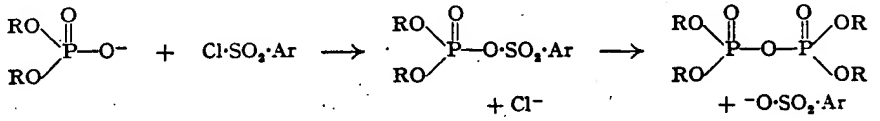
One characteristic of the "exchange reaction" is clear from the preceding account of a single case. Through it an anhydride of a diester of phosphoric acid (*i.e.*, a tetraester of pyrophosphoric acid) is converted into a less reactive anhydride. Thus tetraphenyl pyrophosphate must be stored in sealed containers to prevent its hydrolysis by atmospheric moisture, whereas tetrabenzyl pyrophosphate may be prepared by the reaction between dibenzyl phosphite, carbon tetrachloride, and aqueous alkali (Atherton and Todd, *J.*, 1947, 674) and it was isolated throughout the work described in this paper by solidification under aqueous ammonia at pH 8. The intermediate dibenzyl diphenyl pyrophosphate is correspondingly intermediate in its resistance to hydrolysis. A second point also emerges from the results presented above. The diphenyl phosphate anion must be considerably more stable in acetonitrile solution than the dibenzyl phosphate anion, otherwise the equilibrium between the two anions would permit the "exchange reaction" to proceed in presence of only catalytic quantities of base. In other words, diphenyl hydrogen phosphate must be a stronger acid than dibenzyl hydrogen phosphate and in consequence the anhydrides

derived from them have corresponding relative reactivities. To test the validity of these conclusions we determined by electrometric titration the pK's of diphenyl and dibenzyl hydrogen phosphates in 50% aqueous ethanol. The values so obtained (1·84 and 1·87 respectively) differ by scarcely more than the experimental error. In non-aqueous solvents the acids would be expected to be much weaker and the differences to be more pronounced. The exact determination of acid dissociation constants in non-aqueous media is difficult, but in closely related series of compounds the strengths of acids are closely related to the conductivities of their solutions (cf. Verhoek, *J. Amer. Chem. Soc.*, 1936, **58**, 2577). Accordingly we measured the electrical resistance of decimolar solutions of diphenyl and dibenzyl hydrogen phosphate in acetonitrile and dimethylformamide. The resistance of the cell was lowered from 265 000 ohms with pure acetonitrile to 30 000 ohms by the first compound and to 87 000 ohms by the second, and from 309 000 ohms with pure dimethylformamide to 4400 and 22 200 ohms respectively. In these solvents (*i.e.*, those in which the "exchange reaction" is normally carried out), therefore, diphenyl hydrogen phosphate is a considerably stronger acid than dibenzyl hydrogen phosphate: its anion is, therefore, correspondingly more stable and its anhydride less stable. Further, decimolar solutions of di-*p*- and di-*o*-methoxyphenyl hydrogen phosphates in dimethylformamide had resistances of 5800 and 12 700 ohms respectively: their triethylammonium salts "exchanged" with tetraphenyl but not with tetrabenzyl pyrophosphate. Di-*p*-nitrophenyl hydrogen phosphate, on the other hand, gave a resistance in dimethylformamide under the same conditions of 700 ohms and had a pK of 1·72 in 50% aqueous ethanol. No evidence of its triethylammonium salt "exchanging" with tetraphenyl pyrophosphate could be found. The anhydride derived from this acid should be a powerful reagent and, partly with the intention of completing the series of examples of the "exchange reaction," we attempted to prepare it. Di-*p*-nitrophenyl hydrogen phosphate dissolved slowly in a boiling chloroform solution of thionyl chloride and evaporation of the solvent left a yellow solid. Although this gave, with *cyclo*hexylamine, *cyclo*hexylammonium di-*p*-nitrophenyl phosphate and di-*p*-nitrophenyl *cyclo*hexylaminophosphonate, it was not tetra-*p*-nitrophenyl pyrophosphate. It contained sulphur, liberated sulphur dioxide on hydrolysis, and underwent no "exchange reaction" with triethylammonium diphenyl phosphate: it is presumably analogous in structure to the compound produced from dibenzyl hydrogen phosphate and oxalyl chloride (Mason and Todd, *loc. cit.*). Nor could the pyrophosphate be prepared from the reaction mixture of *p*-nitrophenol, phosphoryl chloride, and pyridine, although this did give di-*p*-nitrophenyl *cyclo*hexylaminophosphonate with *cyclo*hexylamine and di-*p*-nitrophenyl hydrogen phosphate on alkaline hydrolysis.

The elucidation of the nature of this "exchange reaction" between diesters of phosphoric acid and tetraesters of pyrophosphoric acid enabled us to reinterpret the results of some earlier work. This had been directed towards the preparation of mixed anhydrides of toluene-*p*-sulphonic acid and phosphoric acids, such as dibenzyl toluene-*p*-sulphonyl phosphate. Preliminary studies with silver salts in heterogeneous media were unsatisfactory and we turned to the reaction between lithium dibenzyl and diphenyl phosphates and toluene-*p*-sulphonyl chloride in dimethylformamide solution. It was possible to follow the progress of reaction very conveniently by the decrease in electrical resistance of the solution with time as lithium chloride was eliminated. In some twenty minutes the resistance had reached a constant value but this was never as low as would be expected for complete reaction. Moreover, after addition of ammonia to the reaction mixture, the appropriate aminophosphonate, phosphoric acid, and toluene-*p*-sulphonamide were all isolated. Initially we had thought that this mixture of products had arisen from cleavage of the mixed anhydride in two directions. The same products would, however, arise if the mixed anhydride reacted rapidly with more lithium salt to give a pyrophosphate and thus half the toluene-*p*-sulphonyl chloride remained unchanged. This explanation accords better with the magnitude of the drop in electrical resistance of the solution. Since the arysulphonic acids are much stronger acids than the diesters of phosphoric acid, the intermediate anhydride would be expected to be much more susceptible to "exchange reactions."

In confirmation of these views tetrabenzyl pyrophosphate could be isolated after reaction between triethylammonium dibenzyl phosphate and toluene-*p*-sulphonyl chloride in aceto-

nitrile. The more reactive *p*-nitrobenzenesulphonyl chloride was found to be a preferable reagent however, and gave an 80% yield of tetrabenzyl pyrophosphate. This reagent was also evidently able to form the anhydrides of di-*o*-methoxyphenyl hydrogen phosphate and even di-*p*-nitrophenyl hydrogen phosphate, for the corresponding *cyclo*hexylaminophosphonates were obtained after treatment of the reaction products with *cyclo*hexylamine.



The expansion of the scope of the " exchange " type of reaction to include the formation of anhydrides of diesters of phosphoric acid by means of arylsulphonyl chlorides promoted the search for alternative reagents. Trifluoroacetic anhydride appeared to be attractive. It has been used in the esterification of carboxylic acids, and mixed anhydrides have been postulated as intermediates in the reaction (Bourne, Stacey, Tatlow, and Tedder, *J.*, 1949, 2976). It was indeed found to be an effective reagent for the preparation of tetrabenzyl and tetraphenyl pyrophosphates, although, unlike *p*-nitrobenzenesulphonyl chloride, it did not react with triethylammonium di-*p*-nitrophenyl phosphate. The milder character of trifluoroacetic anhydride was shown in another way. Whereas the intermediate dibenzyl *p*-nitrobenzenesulphonyl phosphate could not be detected in the reaction mixtures of dibenzyl pyridinium phosphate and the sulphonyl chloride, the corresponding process with trifluoroacetic anhydride appeared to lead to dibenzyl trifluoroacetyl phosphate; for on addition of *cyclo*hexylamine no *cyclo*hexylaminophosphonate could be obtained, and if this same type of reaction was carried out in presence of one mol. of diphenyl phosphate anion addition of *cyclo*hexylamine gave an 80% yield of *cyclo*hexylaminophosphonate, *i.e.*, dibenzyl diphenyl pyrophosphate had presumably been formed from the intermediate dibenzyl trifluoroacetyl phosphate. Rather surprisingly the dimethylformamide–sulphur trioxide complex (cf. Kenner, *Chem. and Ind.*, 1951, 14) proved quite inert in reaction with lithium diphenyl and dibenzyl phosphates in dimethylformamide.

If the monobenzyl esters of mononucleotides are comparable in acid strength to dibenzyl hydrogen phosphate, this work with simple model substances has reached the original aim of the investigation, the discovery of a possible route to the dibenzyl esters of the dinucleotide coenzymes avoiding intermediate chlorophosphonates. Symmetrical tetraesters of pyrophosphoric acid can be prepared in high yield from diesters of phosphoric acid by means of *p*-nitrobenzenesulphonyl chloride. The use of the milder reagents, trifluoroacetic anhydride and tetraphenyl pyrophosphate, affords in addition the possibility of preparing unsymmetrical tetraesters. It has not been possible to isolate such unsymmetrical tetraesters in these model experiments since all the pyrophosphates studied have rather similar properties and the reactions cannot be expected to be free of by-products. It may be easier to demonstrate the formation of unsymmetrical tetraesters in the more complex nucleotide field. In considering the application of these results to nucleotide synthesis one practical point must be borne in mind. In our experience dimethylformamide, the preferred solvent for the nucleotides of high molecular weight, is by no means so satisfactory a solvent as acetonitrile. Pyrophosphates and similar compounds seem to react in some way with dimethylformamide and yields are consequently reduced.

Experimental

Tetraphenyl Pyrophosphate.—To a solution of diphenyl hydrogen phosphate (2·07 g.) in dry benzene (40 c.c.) diphenyl chlorophosphonate (1·72 c.c.) was added, followed by pyridine (0·67 c.c.). The reaction mixture was stirred magnetically at room temperature during 6 hours, moisture being rigidly excluded. Precipitated pyridine hydrochloride (0·87 g., 91%) was removed by rapid filtration through sintered glass, and benzene was removed from the filtrate by " freeze drying." The residual yellowish oil was distilled in a short-path still at 3×10^{-4} mm. Tetraphenyl pyrophosphate distilled at 150—155° as a colourless viscous oil (3·58 g., 90%), n_D^{20} 1·5610 (Found : C, 59·7; H, 4·3. Calc. for $C_{24}H_{20}O_7P_2$: C, 59·8; H, 4·2%).

Dibenzyl Diphenyl Pyrophosphate.—Dibenzyl hydrogen phosphate (0·287 g., 1 mol.) was dissolved in dry benzene (10 c.c.) and pyridine (0·08 c.c., 1 mol.) was added. This solution was slowly added to a stirred solution of diphenyl chlorophosphonate (0·22 c.c., 1 mol.) in dry benzene (5 c.c.). Pyridine hydrochloride began to separate after a few minutes and after 3 hours' stirring at room temperature the mixture was filtered and solvent removed by "freeze drying." The crude dibenzyl diphenyl pyrophosphate so obtained was a slightly opalescent viscous resin; on attempted distillation it decomposed and polymerised at *ca.* 165°. That this material consisted essentially of the mixed pyrophosphate was shown by the following experiments. Excess of *cyclo*hexylamine was added to a solution of the material in benzene. A crystalline precipitate rapidly formed and after 3 hours the mixture was filtered. The residue, which was water-soluble, appeared to be *cyclo*hexylammonium diphenyl phosphate. The filtrate was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated. Recrystallised from hexane the residue gave dibenzyl *cyclo*hexylaminophosphonate, m. p. 78—80° undepressed on admixture with an authentic specimen; the yield was 90% calculated on the assumption that the starting material was dibenzyl diphenyl pyrophosphate.

That the crude mixed pyrophosphate contained only a small amount of tetrabenzyl pyrophosphate was shown by keeping it in contact with aqueous ammonia at pH 8 for 6 hours (the pH being maintained by addition of more ammonia at intervals). Of the possible pyrophosphates present only tetrabenzyl pyrophosphate is stable under these conditions. The residue from 0·85 g. of material treated in this way was only 0·15 g. of impure tetrabenzyl pyrophosphate (m. p. 51—58°).

Di-p-*methoxyphenyl Hydrogen Phosphate.*—Pyridine (7·3 c.c.) was added slowly to a stirred solution of *p*-methoxyphenol (7·5 g.) and freshly distilled phosphoryl chloride (2·8 c.c.) in dry benzene (40 c.c.); heat was evolved and pyridine hydrochloride began to separate almost at once. The mixture was stirred at room temperature for 10 hours, then filtered, and the filtrate was evaporated. The yellow oily residue was dissolved in chloroform (40 c.c.) and extracted with 5% aqueous sodium hydroxide (5 × 20 c.c.). The alkaline layer was separated and acidified and the precipitated acid was extracted with chloroform (6 × 20 c.c.). The chloroform extracts were combined, dried (Na_2SO_4), and evaporated. The light brown residue was recrystallised from chloroform–light petroleum, yielding colourless needles of *di*-p-*methoxyphenyl hydrogen phosphate*, m. p. 92—94° (3·8 g., 41%) (Found: C, 54·0; H, 4·8. $C_{14}H_{15}O_6P$ requires C, 54·2; H, 4·85%).

Di-p-*methoxyphenyl Diphenyl Pyrophosphate.*—Di-*p*-methoxyphenyl hydrogen phosphate (2·21 g., 1 mol.) was dissolved in dry benzene (50 c.c.), and diphenyl chlorophosphonate (1·48 c.c., 1 mol.) was added, followed by pyridine (0·58 c.c., 1 mol.). The mixture was stirred for 8 hours at room temperature in absence of moisture, pyridine hydrochloride (91% of theory) filtered off, and the solvent removed by "freeze drying." Crude di-*p*-methoxyphenyl diphenyl pyrophosphate remained as a viscous oil (4·24 g.).

A portion (2·2 g.) of the product was treated with liquid ammonia, whereupon it became yellow and solidified. Excess of ammonia was allowed to evaporate and the residue was washed with water, giving a solid water-insoluble residue (0·84 g.). Recrystallisation from chloroform gave di-*p*-methoxyphenyl aminophosphonate, m. p. 181—183° undepressed by an authentic specimen (see below) (69%, calculated on mixed pyrophosphate).

Distillation of the crude pyrophosphate in a short-path still began at 150°/10^{-4} mm. and the main bulk of product distilled at 150—165° as a colourless oil. When treated with aniline, this gave a mixture of anilinophosphonates (m. p. 95—115°), together with a mixture of anilinium diphenyl and di-*p*-methoxyphenyl phosphates. Quantitative separation of the components was difficult but they appeared to be present in approximately equal proportions. Evidently extensive dismutation to tetraphenyl and tetra-*p*-methoxyphenyl pyrophosphates had occurred on distillation.

Di-o-*methoxyphenyl Hydrogen Phosphate.*—Prepared as described by Auger and Dupuis (*Compt. rend.*, 1908, **146**, 1152), the acid had m. p. 95—96° (Found: C, 53·9; H, 5·0. Calc. for $C_{14}H_{15}O_6P$: C, 54·2; H, 4·85%). Its cyclo*hexylamine* salt crystallised from ethanol in colourless needles, m. p. 161—162° (Found: C, 58·8; H, 6·7; N, 3·4. $C_{20}H_{28}O_6NP$ requires C, 58·7; H, 6·9; N, 3·4%).

Di-p-*nitrophenyl Hydrogen Phosphate.*—*p*-Nitrophenol (12·9 g., 2 mols.) and phosphoryl chloride (4·2 c.c., 1 mol.) were dissolved in a mixture of acetonitrile (15 c.c.) and dry benzene (120 c.c.). Pyridine (11·5 c.c., 3 mols.) was then added gradually to the cooled and stirred solution during 20 minutes. Stirring was continued at room temperature for 5 hours, then the white precipitate was filtered off, the filtrate evaporated, and the residue dissolved in chloroform.

Aqueous sodium hydroxide (40 c.c. of 5%) was added, the mixture shaken vigorously, and the yellow precipitate of sodium di-*p*-nitrophenyl phosphate was collected. The crude salt was dissolved in warm water (*ca.* 100 c.c.) and filtered from a small amount of tri-*p*-nitrophenyl phosphate (14%; m. p. 157—159° after recrystallisation from acetic acid). The filtrate was made acid to Congo-red, and the pale yellow precipitate of di-*p*-nitrophenyl hydrogen phosphate was recrystallised from ethyl acetate (yield, 7·8 g., 50%; m. p. 176—178°) (Hoeflake, *Rec. Trav. chim.*, 1916, **36**, 58, gives m. p. 175°).

Di-p-*nitrophenyl* cyclo*Hexylaminophosphonate.*—In the above preparation of di-*p*-nitrophenyl hydrogen phosphate there was some evidence that a considerable amount of the corresponding chlorophosphonate was produced. To confirm this, a separate experiment was carried out starting with 3·7 g. of *p*-nitrophenol in which the chloroform solution of the reaction product was treated with *cyclo*hexylamine (4·9 c.c.), the mixture being externally cooled. After 1 hour the mixture was washed thoroughly with hydrochloric acid, water, and aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Recrystallisation of the yellow residue (3·1 g.) first from chloroform–light petroleum and then from benzene gave *di*-p-*nitrophenyl* cyclo*hexylaminophosphonate* as yellow needles, m. p. 174—176° (Found: C, 51·2; H, 4·9; N, 10·2. $C_{18}H_{20}O_4N_3P$ requires C, 51·3; H, 4·75; N, 10·0%).

Action of Thionyl Chloride on Di-p-nitrophenyl Hydrogen Phosphate.—Thionyl chloride (5 c.c.) was added to a suspension of di-*p*-nitrophenyl hydrogen phosphate (0·758 g., dried over phosphoric oxide at 0·1 mm.) in chloroform (25 c.c.), and the mixture was heated under reflux for 19 hours. The clear solution was evaporated to dryness under reduced pressure, yielding a yellow solid which liberated sulphur dioxide on exposure to moisture. This solid was suspended in dry chloroform (20 c.c.), and *cyclo*hexylamine (0·54 c.c.) was added, practically everything dissolving. During 6 hours at room temperature *cyclo*hexylammonium di-*p*-nitrophenyl phosphate (0·29 g.) separated as pale yellow needles; these were filtered off and the filtrate was washed first with hydrochloric acid, then with aqueous sodium hydrogen carbonate, dried, and evaporated. The residue (0·37 g.), recrystallised from benzene, gave di-*p*-nitrophenyl *cyclo*hexylaminophosphonate, m. p. and mixed m. p. 174—176°. A portion of the yellow solid product from another experiment was treated with *cyclo*hexylammonium diphenyl phosphate in the hope of effecting an exchange. No reaction appeared to take place, since treatment of the reaction mixture with *cyclo*hexylamine yielded only di-*p*-nitrophenyl *cyclo*hexylaminophosphonate.

From its general behaviour it seems possible that the yellow solid obtained in these experiments consists mainly of the mixed anhydride $(NO_2{\cdot}C_6H_4{\cdot}O)_2P(O){\cdot}O{\cdot}SO{\cdot}O{\cdot}P(O){\cdot}(O{\cdot}C_6H_4{\cdot}NO_2)_2$.

Exchange Reactions of Pyrophosphates.—(1) *Effect of amount of added base and variation of solvent.* In this group of experiments the reaction studied was that between tetraphenyl pyrophosphate and dibenzyl hydrogen phosphate to give tetrabenzyl pyrophosphate which is comparatively stable in aqueous media and can readily be isolated. In experiment (*a*) the general procedure adopted for estimating the tetrabenzyl pyrophosphate obtained is indicated; unless otherwise stated it was followed in all other examples.

(*a*) Dibenzyl hydrogen phosphate (0·724 g., 2 mols.) was dissolved in dry acetonitrile (4 c.c.), and triethylamine (0·35 c.c., 2 mols.) added. The solution was now added to tetraphenyl pyrophosphate (0·618 g., 1 mol.) in acetonitrile (1 c.c.), and the whole shaken thoroughly. After 1 hour at room temperature the solvent was removed under reduced pressure. Dilute aqueous ammonia was then added to the oily residue until pH 8 was reached. When scratched with a glass rod the oil solidified and after 30 minutes it was collected (0·545 g., 79%; m. p. 58—60°). After one recrystallisation from ether–*cyclo*hexane the product had m. p. 59—61°, undepressed on admixture with authentic tetrabenzyl pyrophosphate. For further confirmation a portion of the product was dissolved in dimethylformamide, and excess of aqueous ammonia (*d* 0·880) was added. On dilution with water colourless needles of dibenzyl aminophosphonate (m. p. and mixed m. p. 102°) separated.

(*b*) Tetraphenyl pyrophosphate (0·48 g., 1 mol.), dibenzyl hydrogen phosphate (0·557 g., 2 mols.), and triethylamine (0·42 c.c., 3 mols.) reacted under the same conditions to give tetrabenzyl pyrophosphate (0·372 g., 70%).

(*c*) Reaction as in (*b*), but using 4 mols. of triethylamine, gave 54% of tetrabenzyl pyrophosphate.

(*d*) As above but using only 0·1 mol. of triethylamine. No tetrabenzyl pyrophosphate could be isolated. The reaction product became acid (pH *ca.* 2) on addition of water and only a trace of oil (*ca.* 5 mg.) was obtained insoluble at pH 8; this may have been dibenzyl diphenyl pyrophosphate [see (*f*) below].

(*e*) As above, but in absence of any base. No reaction occurred since all the products dissolved in water giving a strongly acid solution.

(*f*) As above using 1 mol. of triethylamine. In this case no tetrabenzyl pyrophosphate was isolated but, instead, an oil was obtained which did not solidify in contact with aqueous ammonia at pH 8 in 6 hours. This oil was mainly dibenzyl diphenyl pyrophosphate as was shown by the following method. After the reactants had been mixed and set aside for 45 minutes the solvent was evaporated and benzene was added to the oily residue, followed by *cyclo*hexylamine (2 mols.). After 2 hours the crystalline precipitate of *cyclo*hexylammonium diphenyl phosphate (80%; m. p. and mixed m. p. 198—199°) was filtered off and the filtrate was washed with dilute hydrochloric acid and water, dried, and evaporated. The solid residue recrystallised from hexane gave dibenzyl *cyclo*hexylaminophosphonate, m. p. and mixed m. p. 78—80°. The yield was 72%, calculated on the assumption that the reaction product was dibenzyl diphenyl pyrophosphate; in another experiment using *cyclo*hexylamine (1 mol.) in place of triethylamine (1 mol.), and dimethylformamide in place of acetonitrile, the yield rose to 81%.

(*g*) As above using 2 mols. of triethylamine but with benzene as solvent. The yield of tetrabenzyl pyrophosphate was 64%.

(*h*) Dry lithium dibenzyl phosphate (0·690 g., 2 mols.) was dissolved in dimethylformamide (8 c.c.), and tetraphenyl pyrophosphate (0·587 g., 1 mol.) in dimethylformamide (2 c.c.) was added to the slightly turbid solution. The turbidity disappeared in 10 minutes, and after a further 50 minutes at room temperature the mixture was worked up in the usual way giving tetrabenzyl pyrophosphate (0·461 g., 71%).

(2) *Effect of acid strength.* The general method employed is set out in experiment (*a*) and, except where otherwise stated, proportions of reactants and conditions were similar throughout, the products being identified by reaction with ammonia to give aminophosphonates.

(*a*) Tetraphenyl pyrophosphate (0·64 g., 1 mol.) was added to dibenzyl triethylammonium phosphate [0·649 g. (2 mols.) of dibenzyl hydrogen phosphate + 0·2 c.c. (2 mols.) of triethylamine] in anhydrous dimethylformamide (2 c.c.) at room temperature. After 5—10 minutes aqueous ammonia (10 c.c.; *d* 0·880) was added, and the mixture shaken vigorously, set aside for 1 hour, and diluted with water to *ca.* 25 c.c. The precipitated dibenzyl aminophosphonate (0·175 g., 50% calculated on tetrabenzyl pyrophosphate) was recrystallised from carbon tetrachloride and had m. p. and mixed m. p. 102—103°.

(*b*) Tetraphenyl pyrophosphate (1 mol.) and di-*o*-methoxyphenyl triethylammonium phosphate (2 mols.), treated in the same way, gave *di-o-methoxyphenyl aminophosphonate*, crystallising from chloroform in colourless needles (90%), m. p. 68—70° (Found: C, 54·6; H, 5·4; N, 4·6. $C_{14}H_{16}O_5NP$ requires C, 54·4; H, 5·2; N, 4·5%).

(*c*) Tetraphenyl pyrophosphate reacted in the same way with di-*p*-methoxyphenyl triethylammonium phosphate, and the mixture treated with ammonia as before, gave *di-p-methoxyphenyl aminophosphonate* (58%), needles, m. p. 179—181° (from chloroform) (Found: C, 54·2; H, 5·1; N, 4·7. $C_{14}H_{16}O_5NP$ requires C, 54·4; H, 5·2; N, 4·5%).

(*d*) Tetraphenyl pyrophosphate did not appear to react with di-*p*-nitrophenyl triethylammonium phosphate under these conditions, since subsequent treatment with ammonia yielded only diphenyl aminophosphonate (48%; m. p. and mixed m. p. 147—148°).

Relative Acid Strengths of Some Diesters of Phosphoric Acid in Various Solvents.—Measurements of pK's for several diesters used in this work were made in aqueous and aqueous-ethanolic solution by electrometric titration (cf. Kumler and Eiler, *J. Amer. Chem. Soc.*, 1943, **65**, 2355).

Since the majority of exchange reactions were carried out in dimethylformamide or acetonitrile or in a mixture of both, the conductivity of solutions of the various diesters of phosphoric acid in these solvents at 25° was determined.

The dimethylformamide used was purified by first storing it over solid potassium hydroxide for 24 hours, then distilling it azeotropically with benzene, and finally fractionating it under reduced pressure. It had b. p. 50—52°/15—16 mm., n_D^{19} 1·4302.

Acetonitrile was dried for 24 hours over anhydrous potassium carbonate, then refluxed with a little phosphoric oxide, decanted, and distilled from a fresh small quantity of the same desiccant. It had b. p. 82°/760 mm.

Diphenyl hydrogen phosphate which normally crystallised as the dihydrate was dried by heating the molten acid for 12 hours at 80°/0·1 mm. over phosphoric acid and then recrystallising the anhydrous product from dry chloroform–light petroleum; it then had m. p. 68—70° and was kept in a dry atmosphere.

Reaction between Lithium Diphenyl Phosphate and Toluene-p-sulphonyl Chloride.—An approximate measure of the rate of reaction between equivalent quantities of these compounds in

dimethylformamide was obtained by following the change in resistance of the mixture with time, using the circuit described by Woolf (*Chem. and Ind.*, 1950, 544). The resistance of the various reaction components was first determined in dimethylformamide with the following results (all concentrations 0·04 molar; temp. of cell, 20°).

	Resistance (ohms)		Resistance (ohms)
Pure dimethylformamide (solvent) ...	76 500	Lithium toluene-*p*-sulphonate	90
Lithium dibenzyl phosphate	922	Lithium chloride	72
Toluene-*p*-sulphonyl chloride	196		

After mixing of the reactants the resistance decreased rapidly and acquired a constant value (81·5 ohms) after 20 minutes. A similar rate was observed for the reaction between lithium di-*p*-nitrophenyl phosphate and diphenyl chlorophosphonate, but it required about 100 minutes to reach a constant resistance in the reaction between lithium diphenyl phosphate and 2 : 4 : 6-tribromobenzoyl chloride.

A solution of lithium diphenyl phosphate (2·56 g., 1 mol.) in anhydrous dimethylformamide (40 c.c.) was added to toluene-*p*-sulphonyl chloride (1·9 g., 1 mol.) in the same solvent (10 c.c.). After 2 hours at room temperature, gaseous ammonia was passed through the solution till saturated, and the mixture left for 3 hours, then evaporated under reduced pressure. The residue was extracted with chloroform (2 × 25 c.c.) and the combined chloroform extracts were filtered and extracted, first with saturated aqueous sodium hydrogen carbonate (extract A), then with 4% aqueous sodium hydroxide (extract B). The chloroform layer, dried and evaporated, gave a residue (1·41 g.) which, when recrystallised from chloroform, had m. p. 150—151° alone or mixed with authentic diphenyl aminophosphonate (yield, 46% calculated on the basis of mixed anhydride formation, or 92% calculated on tetraphenyl pyrophosphate formation).

Extract A was acidified and extracted with chloroform. The chloroform layer, dried and evaporated, gave diphenyl hydrogen phosphate (1·32 g., 46%). Extract B was acidified and evaporated to dryness, and the residue extracted with absolute ethanol (75 c.c.). When evaporated, the ethanolic extract gave toluene-*p*-sulphonamide (0·191 g., 20%), m. p. 133—134° undepressed by an authentic specimen.

*Tetrabenzyl Pyrophosphate from Toluene-*p*-sulphonyl Chloride and Salts of Dibenzyl Hydrogen Phosphate.*—To a solution of dibenzyl hydrogen phosphate (0·56 g., 1 mol.) in dry benzene (20 c.c.), toluene-*p*-sulphonyl chloride (0·382 g., 1 mol.) was added, followed by dry pyridine (0·16 c.c., 1 mol.). The mixture was stirred at room temperature for 1½ hours, whereupon it became turbid. After a further 3½ hours it was filtered and the solvent removed by "freeze drying," giving a semi-solid residue (0·952 g.). This residue was dissolved in acetonitrile (5 c.c.), and the solution filtered and evaporated. The oily residue was then treated with dilute aqueous ammonia to pH 8 in the usual manner, and yielded tetrabenzyl pyrophosphate (28%), m. p. and mixed m. p. 59—61°.

In a second experiment using triethylamine in place of pyridine, and with acetonitrile as solvent instead of benzene, the yield of isolated tetrabenzyl pyrophosphate was 47%.

Preparation of Pyrophosphates using p-*Nitrobenzenesulphonyl Chloride.*—(1) *Tetrabenzyl pyrophosphate.* Dibenzyl hydrogen phosphate (0·291 g., 1 mol.), *p*-nitrobenzenesulphonyl chloride (0·221 g., 1 mol.), and pyridine (0·08 c.c., 1 mol.) were brought into reaction in benzene as in the previous experiment. After filtration the benzene solution was divided into two portions A and B. To portion A excess of *cyclo*hexylamine (0·12 c.c.) was added, and, after 4 hours at room temperature, the solution was filtered, washed with dilute hydrochloric acid, dried, and evaporated. The residue was dissolved in warm chloroform, and light petroleum added till crystallisation set in. The product (0·04 g.) had m. p. 133—135° undepressed on admixture with authentic N-cyclo*hexyl*-p-*nitrobenzenesulphonamide*, m. p. 135—137° (Found: C, 50·8; H, 5·9; N, 10·0. $C_{12}H_{16}O_4N_2S$ requires C, 50·8; H, 5·64; N, 9·9%). The mother-liquors from the crystallisation of this product were evaporated. Recrystallised from hexane, the residue yielded dibenzyl *cyclo*hexylaminophosphonate (0·066 g., 70% calculated on the formation of tetrabenzyl pyrophosphate), m. p. and mixed m. p. 79—81°.

Portion B was "freeze dried" and the residue treated with dilute aqueous ammonia to pH 8, yielding tetrabenzyl pyrophosphate (0·095 g., 70%), isolated in the usual way.

In a second experiment, dibenzyl hydrogen phosphate (0·575 g., 2 mols.) and triethylamine (0·28 c.c., 2 mols.) were dissolved in acetonitrile (5 c.c.). The mixture was set aside at room temperature for 1 hour, then evaporated and worked up with dilute ammonia in the usual way,

giving tetrabenzyl pyrophosphate (0·431 g., 80%), m. p. 58—60°. The use of dimethylformamide as solvent in this reaction gave only a 47% yield of tetrabenzyl pyrophosphate.

(2) *Tetra*-p-*nitrophenyl pyrophosphate.* Di-*p*-nitrophenyl triethylammonium phosphate (2 mols.), treated with *p*-nitrobenzenesulphonyl chloride (1 mol.) in 1 : 1 acetonitrile–dimethylformamide in the same manner, gave an oil shown to be essentially the pyrophosphate by treatment with *cyclo*hexylamine (4 mols.) at room temperature; the products were di-*p*-nitrophenyl *cyclo*hexylaminophosphonate (46%; m. p. and mixed m. p. 174—176°) and *cyclo*hexylammonium di-*p*-nitrophenyl phosphate (46%).

(3) *Tetra*-o-*methoxyphenyl pyrophosphate.* The experiment described under (2) above was repeated, substituting di-*o*-methoxyphenyl triethylammonium phosphate for di-*p*-nitrophenyl triethylammonium phosphate. The oily product was identified as the pyrophosphate by treatment with *cyclo*hexylamine, giving *di*-o-*methoxyphenyl* cyclo*hexylaminophosphonate* (80%, calculated on the assumption that the product was pyrophosphate), colourless needles (from hexane), m. p. 92—94° (Found: C, 60·9; H, 6·5; N, 3·6. $C_{20}H_{26}O_5NP$ requires C, 61·4; H, 6·65; N, 3·6%).

Preparation of Pyrophosphates by Use of Trifluoroacetic Anhydride.—(1) *Tetrabenzyl pyrophosphate.* Dibenzyl hydrogen phosphate (0·286 g., 2 mols.) was dissolved in acetonitrile (5 c.c.) and triethylamine (0·14 c.c., 2 mols.) added, followed by trifluoroacetic anhydride (0·07 c.c., 1 mol.; Stacey *et al.*, *J.*, 1949, 2976). The mixture was set aside for 1 hour at room temperature and worked up in the usual way at pH 8, giving tetrabenzyl pyrophosphate, m. p. 60—61° (0·2 g., 75%).

(2) *Tetraphenyl pyrophosphate.* The above experiment was repeated using diphenyl hydrogen phosphate in place of dibenzyl hydrogen phosphate. Owing to the instability and difficulty of isolating the tetraphenyl pyrophosphate, the product was assayed by treatment with *cyclo*hexylamine: it gave diphenyl *cyclo*hexylaminophosphonate (64%) and *cyclo*hexylammonium diphenyl phosphate (63%).

(3) *Attempted preparation of tetra*-p-*nitrophenyl pyrophosphate.* Di-*p*-nitrophenyl phosphate (0·49 g., 2 mols.) was dissolved in a mixture of dimethylformamide (3 c.c.) and acetonitrile (7 c.c.), and triethylamine (0·2 c.c., 2 mols.) added, followed by trifluoroacetic anhydride (0·11 c.c, 1 mol.). After 50 minutes the solvent was removed under reduced pressure, the residue taken up in dry chloroform (15 c.c.), and *cyclo*hexylamine (0·35 c.c., 4 mols.) added. The only product isolated was *cyclo*hexylammonium di-*p*-nitrophenyl phosphate, m. p. and mixed m. p. 188—190°; the recovery was 83%.

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224. *The Synthesis of Laminaribiose (3-β-D-Glucosyl D-Glucose) and Proof of its Identity with Laminaribiose isolated from Laminarin.*

By P. BÄCHLI and (the late) E. G. V. PERCIVAL.

Laminaribiose has been synthesised by the interaction of 1 : 2-5 : 6-di*iso*propylidene glucose and tetra-acetyl glucosyl bromide, proof of its formulation as 3-β-D-glucosyl D-glucose being thus obtained. The synthetic material is identical with laminaribiose prepared from laminarin. Various derivatives of the sugar are described including the α- and the β-octa-acetate, hepta-acetyl laminaribiosyl bromide, hepta-acetyl methyl-β-laminaribioside, and methyl-β-laminaribioside.

LAMINARIBIOSE was first studied by Barry (*Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 423), who isolated it as the osazone from the mixture of sugars produced by the action of the juice of *Helix Pomatia* on the polysaccharide laminarin. The free sugar, m. p. 161—162°, was obtained by the partial hydrolysis of laminarin with mineral acid. After removal of the glucose by fermentation and of the oligosaccharides by precipitation with alcohol, the disaccharide was obtained as an amorphous powder which crystallised when its aqueous solution was slowly evaporated. Earlier work by the same author (*ibid.*, 1939, **22**, 59) had shown that laminarin was composed of glucose residues mutually linked through the